

=> s l1 (p) (structure  
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=> s l1 (p) crystal  
L6 23 L1 (P) CRYSTAL

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L7 9 DUPLICATE REMOVE L6 (14 DUPLICATES REMOVED)

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L7 ANSWER 1 OF 9 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN  
AN 2008:490739 BIOSIS  
DN PREV200800490738  
TI High throughput production of recombinant human proteins for  
crystallography.  
AU Gileadi, Opher [Reprint Author]; Burgess-Brown, Nicola A.; Colebrook,  
Steve M.; Berridge, Georgina; Savitsky, Pavel; Smee, Carol E. A.; Loppnau,  
Peter; Johansson, Catrine; Salah, Eidarus; Pantic, Nadia H.  
SO Kobe, B [Editor]; Guss, M [Editor]; Huber, T [Editor]. Methods in  
Molecular Biology, (2008) pp. 221-246. Methods in Molecular Biology.  
Publisher: HUMANA PRESS INC, 999 RIVERVIEW DR, STE 208, TOTOWA, NJ  
07512-1165 USA. Series: METHODS IN MOLECULAR BIOLOGY.  
ISSN: 1064-3745. ISBN: 978-1-58829-809-6(H).  
DT Book; (Book Chapter)  
LA English  
ED Entered STN: 3 Sep 2008  
Last Updated on STN: 3 Sep 2008  
AB This chapter presents in detail the process used in high throughput  
bacterial production of recombinant human proteins for crystal  
structure determination. The core principles are: (1) Generating at least  
10 truncated constructs from each target gene. (2) Ligation-independent  
cloning (LIC) into a bacterial expression vector. All proteins are  
expressed with an N-terminal, TEV protease cleavable  
fusion peptide. (3) Small-scale test expression to identify constructs  
producing soluble protein. (4) Liter-scale production in shaker flasks.  
(5) Purification by Ni-affinity chromatography and gel filtration. (6)  
Protein characterization and preparation for crystallography. The chapter  
also briefly presents alternative procedures, to be applied based on  
specific knowledge of protein families or when the core protocol is  
unsatisfactory. This scheme has been applied to more than 550 human  
proteins (>10,000 constructs) and has resulted in the deposition of 112  
unique structures. The methods presented do not depend on specialized  
equipment or robotics; hence, they provide an effective approach for  
handling individual proteins in a regular research laboratory

L7 ANSWER 2 OF 9 MEDLINE on STN DUPLICATE 1  
AN 2008374571 MEDLINE  
DN PubMed ID: 18542867  
TI High throughput production of recombinant human proteins for  
crystallography.  
AU Gileadi Opher; Burgess-Brown Nicola A; Colebrook Steve M; Berridge  
Georgina; Savitsky Pavel; Smee Carol E A; Loppnau Peter; Johansson

Catrine; Salah Eidarus; Pantic Nadia H  
 CS The Structural Genomics Consortium, Botnar Research Centre, University of  
 Oxford, Oxford, UK.  
 SO Methods in molecular biology (Clifton, N.J.), (2008) Vol. 426, pp. 221-46.  
 Journal code: 9214969. ISSN: 1064-3745.  
 CY United States  
 DT Journal; Article; (JOURNAL ARTICLE)  
 LA English  
 FS Priority Journals  
 EM 200808  
 ED Entered STN: 11 Jun 2008  
 Last Updated on STN: 19 Aug 2008  
 Entered Medline: 18 Aug 2008  
 AB This chapter presents in detail the process used in high throughput  
 bacterial production of recombinant human proteins for crystal  
 structure determination. The core principles are: (1) Generating at least  
 10 truncated constructs from each target gene. (2) Ligation-independent  
 cloning (LIC) into a bacterial expression vector. All proteins are  
 expressed with an N-terminal, TEV protease cleavable  
 fusion peptide. (3) Small-scale test expression to identify constructs  
 producing soluble protein. (4) Liter-scale production in shaker flasks.  
 (5) Purification by Ni-affinity chromatography and gel filtration. (6)  
 Protein characterization and preparation for crystallography. The chapter  
 also briefly presents alternative procedures, to be applied based on  
 specific knowledge of protein families or when the core protocol is  
 unsatisfactory. This scheme has been applied to more than 550 human  
 proteins (>10,000 constructs) and has resulted in the deposition of 112  
 unique structures. The methods presented do not depend on specialized  
 equipment or robotics; hence, they provide an effective approach for  
 handling individual proteins in a regular research laboratory

L7 ANSWER 3 OF 9 MEDLINE on STN DUPLICATE 2  
 AN 2008067110 MEDLINE  
 DN PubMed ID: 18221021  
 TI Crystallization and preliminary X-ray analysis of fluorescent protein  
 mBanana.  
 AU Zhou Yangbin; Wu Yifeng; Song Jiaping; Ding Yu; Hu Xiaojian; Zhang Zhihong  
 CS Department of Physiology and Biophysics, Fudan University, 200433 Shanghai  
 PR China.  
 SO Protein and peptide letters, (2008) Vol. 15, No. 1, pp. 113-4.  
 Journal code: 9441434. ISSN: 0929-8665.  
 CY Netherlands  
 DT Journal; Article; (JOURNAL ARTICLE)  
 (RESEARCH SUPPORT, NON-U.S. GOV'T)  
 LA English  
 FS Priority Journals  
 EM 200805  
 ED Entered STN: 29 Jan 2008  
 Last Updated on STN: 10 May 2008  
 Entered Medline: 9 May 2008  
 AB mBanana is a novel monomeric red fluorescent protein mutant. It was  
 cloned and expressed in Escherichia coli with 10 histidine residues at its  
 N-terminal. After cleavage of the His tag by TEV  
 protease, the mBanana was further purified and crystallized by the  
 hanging-drop vapor-diffusion technique. The crystals can diffract to 2.0A  
 resolution and one set of completed data was collected. It showed that  
 the orthorhombic mBanana crystal was in space group P21 with  
 unit cell parameters (48.629, 42.667, 61.714, 90, 111.676, 90) and  
 contained one molecule in one asymmetric unit.

L7 ANSWER 4 OF 9 MEDLINE on STN DUPLICATE 3

AN 2005028473 MEDLINE  
 DN PubMed ID: 15654889  
 TI Comparison of the substrate specificity of two potyvirus proteases.  
 AU Tozser Jozsef; Tropea Joseph E; Cherry Scott; Bagossi Peter; Copeland  
 Terry D; Wlodawer Alexander; Waugh David S  
 CS Department of Biochemistry and Molecular Biology, Research Center for  
 Molecular Medicine, University of Debrecen, Debrecen, Hungary..  
 tozser@indi.biochem.dote.hu  
 SO The FEBS journal, (2005 Jan) Vol. 272, No. 2, pp. 514-23.  
 Journal code: 101229646. ISSN: 1742-464X.  
 CY England: United Kingdom  
 DT (COMPARATIVE STUDY)  
 Journal; Article; (JOURNAL ARTICLE)  
 LA English  
 FS Priority Journals  
 EM 200503  
 ED Entered STN: 19 Jan 2005  
 Last Updated on STN: 2 Mar 2005  
 Entered Medline: 1 Mar 2005  
 AB The substrate specificity of the nuclear inclusion protein a (NIa)  
 proteolytic enzymes from two potyviruses, the tobacco etch virus (TEV) and  
 tobacco vein mottling virus (TVMV), was compared using oligopeptide  
 substrates. Mutations were introduced into TEV protease  
 in an effort to identify key determinants of substrate specificity. The  
 specificity of the mutant enzymes was assessed by using peptides with  
 complementary substitutions. The crystal structure of  
 TEV protease and a homology model of TVMV protease were  
 used to interpret the kinetic data. A comparison of the two structures  
 and the experimental data suggested that the differences in the  
 specificity of the two enzymes may be mainly due to the variation in their  
 S4 and S3 binding subsites. Two key residues predicted to be important  
 for these differences were replaced in TEV protease  
 with the corresponding residues of TVMV protease. Kinetic analyses of the  
 mutants confirmed that these residues play a role in the specificity of  
 the two enzymes. Additional residues in the substrate-binding subsites of  
 TEV protease were also mutated in an effort to alter the  
 specificity of the enzyme.

L7 ANSWER 5 OF 9 MEDLINE on STN DUPLICATE 4  
 AN 2005293963 MEDLINE  
 DN PubMed ID: 15919091  
 TI Crystal structure of tobacco etch virus protease shows the protein C  
 terminus bound within the active site.  
 AU Nunn Christine M; Jeeves Mark; Cliff Matthew J; Urquhart Gillian T; George  
 Roger R; Chao Luke H; Tscuchia Yugo; Djordjevic Snezana  
 CS Department of Biochemistry and Molecular Biology, University College  
 London, Gower Street, London, WC1E 6BT, UK.  
 SO Journal of molecular biology, (2005 Jul 1) Vol. 350, No. 1, pp. 145-55.  
 Journal code: 2985088R. ISSN: 0022-2836.  
 CY England: United Kingdom  
 DT Journal; Article; (JOURNAL ARTICLE)  
 (RESEARCH SUPPORT, NON-U.S. GOV'T)  
 LA English  
 FS Priority Journals  
 EM 200507  
 ED Entered STN: 8 Jun 2005  
 Last Updated on STN: 20 Jul 2005  
 Entered Medline: 19 Jul 2005  
 AB Tobacco etch virus (TEV) protease is a cysteine  
 protease exhibiting stringent sequence specificity. The enzyme is  
 widely used in biotechnology for the removal of the affinity tags from

recombinant fusion proteins. Crystal structures of two TEV protease mutants as complexes with a substrate and a product peptide provided the first insight into the mechanism of substrate specificity of this enzyme. We now report a 2.7Å crystal structure of a full-length inactive C151A mutant protein crystallised in the absence of peptide. The structure reveals the C terminus of the protease bound to the active site. In addition, we determined dissociation constants of TEV protease substrate and product peptides using isothermal titration calorimetry for various forms of this enzyme. Data suggest that TEV protease could be inhibited by the peptide product of autolysis. Separate modes of recognition for native substrates and the site of TEV protease self-cleavage are proposed.

L7 ANSWER 6 OF 9 MEDLINE on STN DUPLICATE 5  
 AN 2003524095 MEDLINE  
 DN PubMed ID: 14601399  
 TI [Tobacco etch virus proteinase: crystal structure of the active enzyme and its inactive mutant].  
 Proteinaza virusa gravirovki tabaka: kristallicheskaia struktura aktivnogo fermenta i ego neaktivnogo mutanta.  
 AU Zhdanov A S; Phan J; Evdokimov A G; Tropea J E; Kapust R B; Li M; Wlodawer A; Waugh D S  
 CS Macromolecular Crystallography Laboratory, Center for Cancer Research, National Cancer Institute at Frederick, MD 21702-1201, United States..  
 zdanov@ncifcrf.gov  
 SO Bioorganicheskaya khimiya, (2003 Sep-Oct) Vol. 29, No. 5, pp. 457-60.  
 Journal code: 7804941. ISSN: 0132-3423.  
 CY Russia: Russian Federation  
 DT (ENGLISH ABSTRACT)  
 Journal; Article; (JOURNAL ARTICLE)  
 LA Russian  
 FS Priority Journals  
 EM 200403  
 ED Entered STN: 7 Nov 2003  
 Last Updated on STN: 2 Mar 2004  
 Entered Medline: 1 Mar 2004  
 AB Tobacco Etch Virus Protease (TEV protease)  
 is widely used as a tool for separation of recombinant target proteins from their fusion partners. The crystal structures of two mutants of TEV protease, active autolysis-resistant mutant TEV-S219D in complex with the proteolysis product, and inactive mutant TEV-C151A in complex with a substrate, have been determined at 1.8 and 2.2 Å resolution, respectively. The active sites of both mutants, including their oxyanion holes, have identical structures. The C-terminal residues 217-221 of the enzyme are involved in formation of the binding pockets S3-S6. This indicates that the autolysis of the peptide bond Met218-Ser219 exerts a strong effect on the fine-tuning of the substrate in the enzyme active site, which results in considerable decrease in the enzymatic activity.

L7 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2008 ACS on STN  
 AN 2003:784050 CAPLUS  
 DN 140:159556  
 TI Tobacco etch virus protease: Crystal structure of the active enzyme and its inactive mutant  
 AU Zhdanov, A. S.; Phan, J.; Evdokimov, A. G.; Tropea, J. E.; Peters, H. K., III; Kapust, R. B.; Li, M.; Wlodawer, A.; Waugh, D. S.  
 CS Center for Cancer Research, Macromolecular Crystallography Laboratory, National Cancer Institute at Frederick, Frederick, MD, 21702-1201, USA  
 SO Russian Journal of Bioorganic Chemistry (Translation of Bioorganicheskaya

Khimiya) (2003), 29(5), 415-418

CODEN: RJBCET; ISSN: 1068-1620

PB MAIK Nauka/Interperiodica Publishing

DT Journal

LA English

AB Tobacco etch virus cysteine protease (TEV protease) is widely used as a tool for the separation of recombinant target proteins from their fusion partners. Here, the crystal structures of 2 mutants of TEV protease, the active autolysis-resistant mutant TEV-S219D in complex with the proteolysis product, and the inactive mutant TEV-C151A in complex with a substrate, were determined at 1.8 and 2.2 Å resolution, resp. The active sites of both mutants, including their oxyanion holes, had identical structures. The C-terminal residues 217-221 of the enzyme were involved in formation of the binding pockets S3-S6. This indicated that the autolysis of the peptide bond Met-218-Ser-219 exerts a strong effect on the fine-tuning of the substrate in the enzyme active site, which results in a considerable decrease in the enzymic activity.

RE.CNT 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD  
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 8 OF 9 MEDLINE on STN DUPLICATE 6

AN 2002733862 MEDLINE

DN PubMed ID: 12377789

TI Structural basis for the substrate specificity of tobacco etch virus protease.

AU Phan Jason; Zdanov Alexander; Evdokimov Artem G; Tropea Joseph E; Peters Howard K 3rd; Kapust Rachel B; Li Mi; Wlodawer Alexander; Waugh David S

CS Macromolecular Crystallography Laboratory, Center for Cancer Research, NCI-Frederick, National Institutes of Health, Frederick, Maryland 21702-1201, USA.

NC N01-CO-56000 (United States NCI)

SO The Journal of biological chemistry, (2002 Dec 27) Vol. 277, No. 52, pp. 50564-72. Electronic Publication: 2002-10-10.  
Journal code: 2985121R. ISSN: 0021-9258.

CY United States

DT (COMPARATIVE STUDY)

Journal; Article; (JOURNAL ARTICLE)

(RESEARCH SUPPORT, NON-U.S. GOV'T)

(RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)

LA English

FS Priority Journals

OS PDB-1LVB; PDB-1LVM

EM 200302

ED Entered STN: 27 Dec 2002

Last Updated on STN: 28 Feb 2003

Entered Medline: 27 Feb 2003

AB Because of its stringent sequence specificity, the 3C-type protease from tobacco etch virus (TEV) is frequently used to remove affinity tags from recombinant proteins. It is unclear, however, exactly how TEV protease recognizes its substrates with such high selectivity. The crystal structures of two TEV protease mutants, inactive C151A and autolysis-resistant S219D, have now been solved at 2.2- and 1.8-Å resolution as complexes with a substrate and product peptide, respectively. The enzyme does not appear to have been perturbed by the mutations in either structure, and the modes of binding of the product and substrate are virtually identical. Analysis of the protein-ligand interactions helps to delineate the structural determinants of substrate specificity and provides guidance for reengineering the enzyme to further improve its utility for biotechnological applications.

L7 ANSWER 9 OF 9 MEDLINE on STN DUPLICATE 7  
 AN 2002329980 MEDLINE  
 DN PubMed ID: 12071693  
 TI A new vector for high-throughput, ligation-independent cloning encoding a tobacco etch virus protease cleavage site.  
 AU Stols Lucy; Gu Minyi; Dieckman Lynda; Raffin Rosemarie; Collart Frank R; Donnelly Mark I  
 CS Environmental Research Division, Argonne National Laboratory, Argonne, IL 60439, USA.  
 NC GM62414-01 (United States NIGMS)  
 SO Protein expression and purification, (2002 Jun) Vol. 25, No. 1, pp. 8-15. Journal code: 9101496. ISSN: 1046-5928.  
 CY United States  
 DT Journal; Article; (JOURNAL ARTICLE)  
 (RESEARCH SUPPORT, U.S. GOV'T, NON-P.H.S.)  
 (RESEARCH SUPPORT, U.S. GOV'T, P.H.S.)  
 LA English  
 FS Priority Journals  
 EM 200212  
 ED Entered STN: 20 Jun 2002  
 Last Updated on STN: 28 Dec 2002  
 Entered Medline: 27 Dec 2002  
 AB To establish high-throughput methods for protein crystallography, all aspects of the production and analysis of protein crystals must be accelerated. Automated, plate-based methods for cloning, expression, and evaluation of target proteins will help researchers investigate the vast numbers of proteins now available from sequenced genomes. Ligation-independent cloning (LIC) is well suited to robotic cloning and expression, but few LIC vectors are available commercially. We have developed a new LIC vector, pMCSG7, that incorporates the tobacco etch virus (TEV) protease cleavage site into the leader sequence. This protease is highly specific and functions under a wide range of conditions. The new vector incorporates an N-terminal his-tag followed by the TEV protease recognition site and a SspI restriction site used for LIC. The vector functioned as expected, giving high cloning efficiencies and strong expression of proteins. Purification and cleavage of a target protein showed that the his-tag and the TEV cleavage site function properly. The protein was purified and cleaved under different conditions to simulate both plate-based screening methods and large-scale purifications for crystal production. The vector also includes a pair of adjacent, unique restriction sites that will allow insertion of additional modules between the his-tag and the cleavage site of the leader sequence to generate a family of vectors suitable for high-throughput production of proteins.  
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